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DMARD AUROTHIOMALATE INHIBITS COX-2, IL-6 AND MMP-3 EXPRESSION IN CHONDROCYTES BY INCREASING MKP-1 EXPRESSION AND DECREASING P38 PHOSPHORYLATION

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Purpose: Aurothiomalate is a disease modifying antirheumatic drug that suppresses inflammation and retards cartilage degradation and bone erosion in arthritis. The molecular mechanisms of action of aurothiomalate are not known in detail. Mitogen-activated protein kinase (MAPK) pathways are major signaling pathways in inflammation, which regulate the production of many factors known to mediate inflammation and cartilage destruction in OA and RA. In the present study we investigated the effects of aurothiomalate on the activity of p38 MAPK and expression of MAPK phosphatase 1 (MKP-1), COX-2, MMP-3 and IL-6 in chondrocytes and intact cartilage.

Methods: Cartilage tissue was obtained from the leftover pieces of total knee replacement surgery from patients with OA and RA and from MKP-1^{-/-} and wild type mice. Immortalized H4 murine chondrocytes* were used in cell culture experiments.

Results: Aurothiomalate inhibited IL-1 β -induced COX-2 expression and prostaglandin E₂ (PGE₂) production by destabilizing COX-2 mRNA, as did the p38 MAPK inhibitor SB203580. Interestingly, aurothiomalate also increased the expression of MKP-1 and reduced IL-1 β -induced phosphorylation of p38 MAPK. Knock-down of MKP-1 by siRNA significantly impaired the ability of aurothiomalate to inhibit the phosphorylation of p38 MAPK and the expression of COX-2, MMP-3 and IL-6. Likewise, aurothiomalate reduced COX-2, MMP-3 and IL-6 expression in human rheumatoid arthritis (RA) and osteoarthritis (OA) cartilage, and in articular cartilage from wild type mice but not in cartilage from MKP-1^{-/-} mice.

Conclusions: The results provide a novel mechanism for the anti-inflammatory and anti-erosive action of aurothiomalate through increased MKP-1 expression, reduced p38 MAPK activation and suppressed expression of COX-2, MMP-3 and IL-6. MKP-1 may therefore be a promising novel target for the development of disease modifying drugs for RA and OA.

*Murine H4 chondrocyte cell line was kindly provided by Peter van der Kraan, Laboratory of Experimental Rheumatology, University Medical Center Nijmegen, Netherlands.

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COLLAGEN PEPTIDE SUPPLEMENTATION STIMULATES PROTEOGLYCAN BIOSYNTHESIS AND AGGREGAN EXPRESSION OF ARTICULAR CHONDROCYTES

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Purpose: Over the past years hydrolyzed collagen has been used in the treatment of OA and positive effects on joint health were demonstrated in pre-clinical experiments and clinical studies. The therapeutic mechanism, however, is still unknown.

The aim of this study was to investigate the influence of a specific Collagen Hydrolysate (FORTIGEL®) on the proteoglycan metabolism of the extracellular matrix (ECM) of chondrocytes.

Methods: Primary articular chondrocytes were isolated of porcine ankle joint cartilage and cultured under reduced oxygen con-

ditions. The culture medium was supplemented with 0.5 mg FORTIGEL®/ml according to recommended daily dose of patients. At different time points of the culture period the amount of secreted and cell-associated proteoglycans (PGs) were quantified by measuring 35S-sulphate incorporation. Total proteoglycan biosynthesis was measured by specific staining of sulphate groups with Alcian blue. Moreover, the expression of aggrecan was determined by Northern Blot analysis and the amount of aggrecan in the ECM was analyzed via Western Blotting.

Results: Supplementation of the culture medium with FORTIGEL® resulted in a statistically significant ($p < 0.05$) increase of total PG synthesis. The amount of secreted and cell-associated PGs was significantly increased up to 1.6-fold after FORTIGEL® treatment compared with the control cells. In particular, administration of FORTIGEL® was associated with a statistically significant increase ($p < 0.05$) of aggrecan biosynthesis shown by RNA expression and accumulation of aggrecan intermediates and of native aggrecan in the ECM as well.

Conclusions: These results indicate a stimulatory effect of FORTIGEL® on the metabolism of proteoglycans in chondrocytes. Thus FORTIGEL® may be helpful reducing degenerative changes of the ECM by stimulating anabolic processes in cartilage tissue.

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THE EFFECTS OF DEOXYNIVALENOL, NIVALENOL, T-2 TOXIN AND SELENIUM SUPPLEMENTATION ON IN VITRO TISSUE ENGINEERED CARTILAGE METABOLISM

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Purpose: To investigate the effects of Nivalenol (NIV), Deoxynivalenol (DON) and T-2 toxin, in the presence and absence of Selenium (Se), on the metabolism of tissue engineered cartilage. These in vitro cartilage cultures mimic those found in Kashin-Beck Disease (KBD) environments that cause diarthrodial joint degeneration in skeletal joint development and osteoarthritis with ageing.

Methods: Bone matrix gelatin (BMG) was prepared from cancellous bones of adult rabbits using previously published procedures (Li et al. J Zhejiang Univ Sci B. 2008, 9(1): 22-33). Human chondrocytes were isolated from a 10th-week human embryo cartilage anlagen by sequential enzyme treatments. One million Passage 2 cells were seeded onto BMG grafts for pre-culture for 48 hours. Three toxins (DON, NIV & T-2 toxin), in the presence or absence of Se, were added: DON, 1.0 μ g/ml; NIV, 0.1 μ g/ml; T-2, 0.01 μ g/ml; Se, 0.1 μ g/ml. After 3 weeks culture in vitro, the BMG with the tissue-engineered cartilage graft was fixed, embedded, and cut into 14 μ m slices for histological analysis. Slides were stained with hematoxylin & eosin (H&E) and toluidine blue. Immunohistochemical analysis was used to detect the expression of types II & X collagen, aggrecan, MMP1, MMP3, TIMP1, TIMP3 and α 2 macroglobulin.

Results: H&E staining showed that the tissue-engineered cartilage grafts were very similar to normal cartilage in vivo, with chondrocytes localised in lacunae, separated from each other by the interterritorial extracellular matrix. There were no necrotic chondrocytes observed in the Control or the Toxin/Se treated groups. Control tissue engineered cartilage grafts expressed aggrecan and type II collagen, and a small amount of type X collagen. Immunohistochemical analysis of grafts exposed to DON, NIV and T-2 toxin showed decreased expression of type II collagen and aggrecan. However, Se addition restored type II collagen and aggrecan staining in the presence of these Toxins. There was increased MMP-1 and MMP-3 expression in the Toxin-treated grafts. How-